COULTER® GEN•S ST™SlideStainer

Optimizing Stain Protocols

Overall Stain Quality

Beckman Coulter has developed BCI TruColor™ reagents and default protocols to be used on the SlideStainer. The Beckman Coulter default protocols have been optimized to a predefined coloration specification (See tables on reverse side). Since coloration is subjective, optimization of the protocols and/or reagents may be necessary to meet your individual coloration criteria. Ensure that both the methanol and water you use meet the specifications detailed in the GEN•S™ Cell Help.

Typical time adjustments increments

- Stain – 30 seconds
- Buffer – 1 minute

Coloration (red / blue) is primarily controlled by the pH of the buffer.

Stain intensity and differential staining characteristics can controlled by:

- the length of staining times (longer = more intense)
- decreasing the buffer strength
- concentration of stain in buffer (more stain = darker, more intense)

Symptom	Cause	Action
Too red	Buffer too acidic	a) Adjust with alkaline solution until the desired color is achieved. b) Try a buffer with a higher pH.
	Excessive washing	a) Shorten rinse time (Bath 5) until the desired color is achieved. b) Adjust frequency of Bath 5 drain and fill until the desired color is achieved.
	All reagents too old	Drain and fill all baths.
	Stain time too short	Prolong staining time until the desired color is achieved.
	Buffer time too long	Shorten buffer time until the desired color is achieved.
Too blue	Buffer too alkaline	a) Adjust with acid solution until the desired color is achieved. b) Try a buffer with a lower pH.
	Insufficient washing	Lengthen rinse time until the desired color is achieved.
	Stain time too long	Decrease stain time until the desired color is achieved.
	Buffer time too short	Lengthen buffer time until the desired color is achieved.
Too dark	Stain time too long	Shorten stain time until the desired color is achieved.
	Buffer time too long	Shorten buffer time until the desired color is achieved.
Too light	Rinse time too long	Decrease rinse time until the desired color is achieved.
	Poor quality rinse water	a) Change water. b) Verify water meets specifications of Type II NCCLS water.
	Stain time too short	Increase stain time until the desired color is achieved.
	Buffer time too short	Increase buffer time until the desired color is achieved.
	Buffer too concentrated.	Dilute buffer solution with deionized or distilled water until the desired color is achieved.
RBC centers appear punched out (cookie cutter effect in center of cells)	Water has contaminated the methanol.	a) Drain/Fill the methanol bath. b) Dip empty basket in methanol prior to re-entry into system.

Background staining (overall slide shows color)	Stain too old	Drain/fill Stain and Stain/Buffer baths.
	Excess stain left on slide	Add quick rinse step to protocol (primarily for WG and MGG). See help topic Quick Rinse Method.
Precipitate (clumps or fine particles on whole slide)	Stain or Stain/buffer too old	Drain/Fill all baths.
	Rinse (Bath 5) dirty	a) Drain/Fill Bath 5. b) Increase frequency of Bath 5 drain/fill. c) Add Quick Rinse step to protocol (primarily for WG or MGG). See help topic Quick Rinse Method.
	Stain baths dirty (Stain and Stain/Buffer)	Perform the Bath Flushing Procedure.
Overall pale blue staining	Blood smear more than 2 hours old prior to fixing	Make new blood smear.

The tables below represent the stain characteristics against which the Beckman Coulter TruColor™ reagents and BCI default protocols were evaluated.

Human Blood Cell Inclusions

Inclusion	Description	Cell	Color
Auer rods	Spicule-like formation	Myeloblast	Pink
Pappenheimer bodies	Small spots	Red blood cells	Purple
Malaria parasites	Inclusions	Red blood cells	Purple/blue
Howell-Jolly bodies	Medium sized spot	Red blood cells	Purple
Cabot rings	Threadlike filaments	Red blood cells	Purple
Basophilic stippling	Small granules	Red blood cells	Purple
Toxic Granulation	Coarse Granules	WBC	Dark Purple
Dohle bodies	Spots	WBC	Sky-Blue

Coloring of Romanowsky Stains

Structure	Cell	Color
Nuclei	All	Purple
Cytoplasm	Erythrocyte	Pink
	Lymphocyte	Blue
	Monocyte	Gray blue
	Neutrophil	Pink
	Metamyelocyte	Pink
	Myelocyte	Pink
	Promyelocyte	Blue
Granules	Basophil	Purple, black
	Eosinophil	Orange
	Neutrophil	Purple
	Platelet	Purple

Reference:
Wintrobe's Clinical Hematology, 9th edition, Vol. 1, page 23.
NCCLS Document H20 A, Volume 12, Number 1. Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental methods.
Color Atlas and Instruction Manual of Peripheral Cell Morphology, O'Connor, Barbara H., 1984, page 16 – 18.

Copyright© Coulter International Corp. 2000
All Rights Reserved
Printed on Recycled Paper
Come visit us at www.beckmancoulter.com

PN 4276448A (July 2000)